Reprinted from Vol. 111(4), July 1986
Journal of the American Society for Horticultural Science
Alexandria, VA 22314

J. AMER. SOC. HORT. SCI. 111(4):618–622. 1986.

Breeding Strategies for Increasing the Anthocyanin Content of Cranberries

Gerald M. Sapers, Susan B. Jones, Michael J. Kelley, and John G. Phillips
Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, PA 19118

Eric G. Stone
Rutgers University Blueberry and Cranberry Research Center, Agriculture Research Service, U.S. Department of Agriculture, Chatsworth, NJ 08019

Additional index words. *Vaccinium macrocarpon*, fruit breeding, cranberry fruit composition

Abstract. **The effect of cultivar on cranberry (*Vaccinium macrocarpon* Ait.) fruit size and composition was investigated. 'Ben Lear', 'Crowley', 'Early Black', and 'Franklin' berries contained about twice the anthocyanin of the other clones. Based on projections of analytical data, potential gain could be enhanced by increasing the proportion of berries that attain high anthocyanin content, seen in individual fruits within samples, as compared to the alternative strategies of breeding for improved anthocyanin content, for small berries, or for synchronous ripening.**

Because the value of cranberries and their products depends largely on color, a manifestation of their anthocyanin content (8, 17), investigators have sought to increase pigmentation by breeding (6, 9) and by the application of growth regulators such as ethephon (3, 5, 7). With the latter approach, cranberries may be stimulated to accumulate anthocyanin up to, but not exceeding, some biological limit (12). The stimulation of anthocyanin biosynthesis by light is well known (11). Light exposure has been shown to increase both anthocyanin accumulation and ethylene production in cranberry (3); however, the relationship between such stimulation and the ripening process is not clear (1). Previous comparisons of various cultivars of cranberries have demonstrated large differences in anthocyanin content. Zukerman et al. (20) observed a 2- to 3-fold range in the anthocyanin content of fruits of 12 cultivars and selections grown in 3 Massachusetts bogs; a large location effect on pigmentation was seen. Schmid (16) reported a 2-fold range in total anthocyanin for 12 cultivars, whereas Weiss et al. (19) obtained a 2.7-fold range for 10 cultivars. The latter investigators reported a 6-fold range for individual samples evaluated over a 5-year period. In a comparison of 21 cultivars grown in Massachusetts in 1980, we observed almost a 4-fold range in total anthocyanin content (unpublished data). More recently, we have investigated variation in the anthocyanin content of 16 cranberry cultivars and selections, relating sample anthocyanin content to the berry size distribution and berry-to-berry variation in surface coloration (13). In the present study, we have used these data to estimate the upper limit to anthocyanin accumulation that might be realized in cranberry fruits of several cultivars under the conditions of this investigation, and to compare several breeding strategies that might be followed to increase anthocyanin accumulation in cranberries. We also investigated whether cultivar-related differences in pigmentation could be explained by structural features.

Received for publication 14 Aug. 1985. Reference to brand or firm name does not constitute endorsement by the USDA over others of a similar nature not mentioned. We acknowledge the technical assistance of Anita M. Burgher, Gavin R. Graff, Sandra P. Graham, and Timothy O. Dobson, employees of the Eastern Regional Research Center. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Materials and Methods

Samples of fruit from 16 cranberry clones were harvested over a period of several days in mid-Oct. 1983 from bogs in Chatsworth, N.J. The clones were classified according to earliness on the basis of previous studies (2, 4, 6; P.E. Marucci, personal communication). Each sample was sorted into 9 subsamples based on berry size and surface coloration. Subsample weight distributions were calculated from the original sample weight and the weight of each new subsample. Details of sorting procedures and all analytical methods are described in a companion paper (13). The subsamples were analyzed for mean berry weight, juice yield, percentage of soluble solids, titratable acidity, and total anthocyanin content (berry, surface, and juice). Weighted mean values for these parameters and for anthocyanin recovery (juice total anthocyanin × juice yield ÷ berry total anthocyanin) were computed for each clone from the subsample analytical values, using the fraction of the total sample weight represented by each subsample as the weighting factor.

$$\text{Weighted mean} = \frac{\Sigma\text{Subsample mean} \times \text{subsample weight}}{\text{sample weight}}$$

Maximum values for the anthocyanin content of the darkest individual berries were used to estimate the biological limit in pigmentation for selected cultivars.

For structural studies, fully ripe, dark-colored berries from 1984 samples of 'Ben Lear', 'Early Black', 'Franklin', 'Howes', 'McFarlin', and 'Searles' cultivars, which differ greatly in anthocyanin content, were examined by light microscopy. Berries of similar coloration and typical size were compared. Segments taken from the equatorial region of fresh berries were fixed in 3% glutaraldehyde, 0.1 M sodium cacodylate buffer, pH 7.0, for 3 hr at room temperature, and rinsed overnight at 4°C in buffer. The tissue then was cut into 2-mm^3 pieces; post-fixed in 1% osmium tetroxide in 0.08 M sodium cacodylate buffer, pH 7.0, for 4 hr at room temperature; dehydrated in a graded series of acetone/water solutions; and embedded in Spurr's low-viscosity resin. Sections were cut 1-mm thick and stained with methylene blue for contrast enhancement. Observations were made of the numbers of pigmented cell layers, the density of cells in each layer, and the depth of the pigmented cell layers. Dimensions of structural features were measured with an eyepiece micrometer.

Potential gains in berry anthocyanin content resulting from

Table 1. Berry size, juice yield, and composition of cranberry clones[z].

Clone	Ripening season	Berry wt (g/berry)	Juice yield (ml/100 g)	Soluble solids (% at 20°)	Titratable acidity (% citric)	Total anthocyanin			
						Berry (mg/100 g)	Juice (mg/100 ml)	Surface[y] (mg·cm^{-2})	Recovery[x] (%)
Franklin	Early	1.4 ab	73 a	9.3 a	2.0 a	43 a	23 a	1.4 a	38 a
Early Black	Early	1.0 b	75 a	9.1 a	2.2 a	43 a	24 a	1.3 ab	41 a
Crowley	Mid-late	1.5 ab	76 a	8.7 a	2.3 a	41 a	24 a	1.2 b	44 a
Ben Lear	Early	1.7 a	71 a	9.5 a	2.1 a	36 ab	20 ab	1.3 ab	40 a
BD[w]	Mid	1.4 ab	78 a	8.3 a	2.0 a	28 abc	14 ab	1.0 c	39 a
McFarlin	Late	1.2 ab	75 a	9.2 a	2.2 a	24 bc	13 ab	0.9 c	38 a
AJ[w]	Mid	1.5 ab	79 a	9.3 a	2.2 a	24 bc	13 ab	0.8 cde	42 a
Pilgrim	Late	1.8 a	77 a	8.3 a	2.3 a	22 bc	11 ab	0.8 cde	39 a
20[v]	Late	1.7 a	78 a	8.6 a	2.2 a	21 c	10 ab	0.9 cd	39 a
Howes	Late	1.2 ab	74 a	9.6 a	2.2 a	20 c	12 ab	0.9 c	38 a
Stevens	Late	1.7 ab	77 a	9.0 a	2.4 a	20 c	10 ab	1.0 c	39 a
CN[w]	Mid	1.6 ab	77 a	8.3 a	2.2 a	20 c	10 ab	0.8 cde	37 a
Searles	Mid	1.5 ab	77 a	9.9 a	2.3 a	18 c	10 ab	0.9 c	44 a
Beckwith	Late	1.6 ab	76 a	9.3 a	2.4 a	18 c	11 ab	0.9 c	47 a
Wilcox	Late	1.2 ab	76 a	9.3 a	2.3 a	17 c	10 ab	0.7 de	42 a
35[v]	Late	1.8 a	76 a	9.0 a	2.2 a	13 c	8 b	0.6 e	45 a
Means	Early	1.4	73	9.3	2.1	40	22	1.4	40
	Mid	1.5	77	8.9	2.2	26	14	0.9	41
	Late	1.5	76	9.0	2.3	20	11	0.8	41

[z]Weighted means; mean separation in columns by Bonferroni t test at $P = 0.05$.
[y]Total anthocyanin in 100 g berries ÷ surface area of 100 g berries; dark-red subsamples only.
[x]Juice anthocyanin × juice yield ÷ berry anthocyanin.
[w]USDA breeding program, 1943 selections (4).
[v]USDA breeding program, 1938–1940 selections (4).

certain assumed changes in the characteristics of the samples were estimated as follows:

a. *Increased surface anthocyanin content*. Calculate surface anthocyanin content for each subsample of the highly colored clones from subsample total anthocyanin contents (TAcy) and corresponding values of the mean berry weight (W) by means of the previously described equation (13):

$$\text{Surface anthocyanin (mg·cm}^{-2}) = \text{TAcy} \times \text{W}^{1/3}/47.1$$

Assume surface anthocyanin contents for dark-, medium-, and light-red subsamples equal to the values obtained with the most highly colored clone. For each clone, calculate the projected anthocyanin content for all subsamples from their assumed surface anthocyanin contents and experimentally determined mean berry weights with the surface anthocyanin equation. Calculate the projected clone anthocyanin content from subsample values and the experimentally determined subsample weight distribution, and compare this value with the actual anthocyanin content.

b. *One-hundred percent small berries*. Assume that all of the berries in a sample are small, as defined by the size separation procedure described in the companion paper (13), with no change in the proportions of dark-, medium-, and light-red berries. Calculate the projected clone anthocyanin content from the measured anthocyanin values for the dark-, medium-, and light-red subsamples and their weight distribution.

c. *One-hundred percent dark berries*. Assume that all of the berries in a sample develop in synchrony and attain the same surface anthocyanin content, which corresponds to the total anthocyanin contents of dark-red small, medium, and large berry subsamples. Calculate the projected clone anthocyanin content from these values and the appropriate subsample weight distribution.

d. *One-hundred percent berries with maximum surface anthocyanin content*. Assume that all of the berries in a sample are similar in anthocyanin content to the individual berry with the greatest surface anthocyanin content. Calculate projected anthocyanin contents for the small, medium, and large berry subsamples from the mean berry weights and assumed surface anthocyanin contents with the equation given previously. Then calculate the projected clone anthocyanin content from the subsample values and weight distribution.

Statistical methods. Comparisons of means were made by application of the Bonferroni t test. All statistical computations were performed with the Statistical Analysis System General Linear Models and Nested Procedures (SAS Institute, Cary, N.C.).

Results and Discussion

Characteristics of cranberry cultivars. Weighted mean values of the berry size and composition parameters for each clone are presented in Table 1. Mean berry weights varied between 1.0 and 1.8 g, reflecting differences in the distribution of small, medium, and large berries in each sample. Mean juice yields and values of the soluble solids content and titratable acidity were similar for the 16 clones and comparable to published composition data (6, 16, 18, 19). Previously, we reported little variation in soluble solids and titratable acidity in a comparison of 45 cultivars and selections (15).

Differences among the samples in berry and juice total anthocyanin contents were large: 'Ben Lear', 'Crowley', 'Early Black', and 'Franklin' berries contained about twice the anthocyanin of other clones. This distinction also held when the pigment content was expressed as surface anthocyanin. Anthocyanin contents were greater in early maturing clones than in those maturing later. Clones generally were similar in anthocyanin recovery, as reported previously (14).

Schmid (16) also reported high anthocyanin contents in 'Early

Table 2. Maximum total anthocyanin content (TAcy) of individual cranberries in dark-red subsamples.

Cultivar	Cultivar TAcy (mg/100 g)	Berries with highest TAcy[z] (Rank)	Berry TAcy (mg/100 g)	Surface TAcy ($mg \cdot cm^{-2}$)
Crowley	41	1	127	2.8
		2	117	2.4
		3	105	2.2
Early Black	43	1	117	2.6
		2	107	2.3
		3	103	2.5
Franklin	43	1	130	2.8
		2	115	3.0
		3	107	2.6
Howes	20	1	71	1.5
		2	70	1.7
		3	68	1.7
McFarlin	24	1	104	2.2
		2	84	1.9
		3	79	1.8
Wilcox	17	1	58	1.3
		2	55	1.2
		3	53	1.3

[z]Individual berries with 3 highest TAcy values from among 20 darkest berries in dark-red subsamples.

Table 3. Potential gain in total anthocyanin content (TAcy) from alternative breeding or production strategies.

Cultivar	Ripening season	Observed TAcy (mg/100 g)		Breeding strategy 1 Assume same surface TAcy as 'Franklin'	2 Assume all small berries	3 Assume all dark berries	4 Assume all[y] berries at max. surface TAcy
		Mean	Maximum[z]				
				Projected TAcy (mg/100 g)			
Crowley	Mid-late	41	127	48	54	48	112
Early Black	Early	43	117	51	49	60	118
Franklin	Early	43	130	43	54	60	126
Howes	Late	20	71	37	26	39	73
McFarlin	Late	24	104	40	30	40	98
Wilcox	Late	17	58	37	23	30	57
				Mean % gain[x]			
All cvs (16)		---	---	168	127	161	322
Early cvs (3)		---	---	111	126	141	286
Mid cvs (5)		---	---	163	125	153	271
Late cvs (8)		---	---	186	129	172	363

[z]For individual berries.
[y]Projected TAcy × 100 ÷ observed TAcy.
[x]Mean percent gain for 2 early season, one midseason, and one late season cultivars only.

Black' and 'Franklin' cranberries and about half as much anthocyanin in 'Beckwith', 'Howes', 'McFarlin', 'Pilgrim', 'Searles', 'Stevens', and 'Wilcox' berries. However, Weiss et al. (19) reported that 'Howes' contained almost as much anthocyanin as 'Early Black' and 'Franklin'. Discrepancies such as this probably arose from the comparison of samples taken at different stages of ripeness or color development. In a 1980 comparison of clones grown at the Univ. of Massachusetts Cranberry Experiment Station in East Wareham (unpublished data), we obtained substantially larger total anthocyanin values for 'Early Black' and 'Franklin' than for 'Howes' and a number of other cultivars and selections that were also compared in the present study. These samples all contained more anthocyanin than did the 1983 New Jersey cranberries. However, the 1980 values were very similar to the values reported by Schmid (16).

The maximum total anthocyanin contents found in the darkest individual New Jersey berries (Table 2), which exceeded cultivar means by at least a factor of 3, represent the upper limit for pigmentation under the specific conditions of this study, e.g., the 1983 season at the Chatsworth location with samples

having a modest anthocyanin content. The degree to which these maximum values approximate a biological limit requires further study with samples representing optimal production conditions and harvest dates. When expressed as surface anthocyanin to compensate for differences in berry size, the maximal anthocyanin contents of these samples were in the same rank order as the total anthocyanin contents of the bulk samples, namely: 'Crowley' = 'Early Black' = 'Franklin' > 'McFarlin' ≥ 'Howes' = 'Wilcox'. We suggest that the 2-fold difference in surface anthocyanin represented by these samples may be indicative of the gain in total anthocyanin that could be realized by breeding for increased pigmentation.

Because our data indicated important differences among clones in anthocyanin content that might be exploited by breeders, we examined berry samples from 1984 to determine whether the anthocyanin content could be related to structural features. 'Ben Lear', 'Early Black', and 'Franklin' berries, which have high anthocyanin contents, were compared with 'Howes', 'McFarlin', and 'Searles' berries, which contain less anthocyanin.

Microscopic observations of cranberry sections taken from dark-colored berries of the 6 cultivars revealed differences among samples in the numbers and dimensions of pigment-bearing cells (epidermal cells and the first cell layer beneath). However, no relationship between structural features, such as the density of pigment-bearing cells in the first 2 cell layers, the thickness of these layers, or the cuticle thickness, and the sample anthocyanin contents could be elucidated. Apparently, differences in anthocyanin content among clones depend on the capacity of the berries to synthesize anthocyanin rather than on differences in the quantity or size of pigmented cells.

Potential genetic gains in anthocyanin content. Increases in the anthocyanin content of cranberries might be realized through breeding, i.e., by selecting for increased pigmentation, for small berries, for early color development, for more synchronous color development, or for various combinations of these characteristics. Estimates of potential gains for various strategies were based on projections from the subsample weight distributions and anthocyanin contents. The first strategy assumes that the high capacity for accumulating anthocyanin seen in highly colored cultivars like 'Franklin' could be bred into selections deficient in this respect. Surface anthocyanin values determined for this cultivar (1.44, 0.80, and 0.33 $mg \cdot cm^{-2}$ for dark-, medium-, and light-red subsamples, respectively) were used as the target of this breeding strategy. Gains for the 2nd strategy were calculated by assuming that the average berry size could be reduced by breeding to that of our small subsamples with no change in the surface anthocyanin content. The 3rd strategy assumes that the cranberries have been selected for synchronous ripening (resulting in synchronous color development) and that all of the berries in each sample were as high in anthocyanin content as our dark subsamples. It is evident from our projections that the potential gain would be greater if the first or 3rd strategies (breeding for higher anthocyanin content or for synchronous color development) were followed (Table 3). However, the potential gain from each of these breeding strategies is much less than the gain that could be realized by fully using the capacity of cranberries to accumulate anthocyanin, i.e., to achieve the maximum anthocyanin content found in individual berries. Our data suggest that a tripling or even quadrupling of the anthocyanin content (mean gain of 363% projected for 8 late-maturing clones) might be possible if all berries could attain the maximum surface anthocyanin values found in these samples. Conceivably, an even higher gain might be obtained if our estimates of the biological limit were low, a distinct possibility in view of the single location sampled and the small number of individual berries examined. We can speculate that the basis for berry-to-berry variation in anthocyanin content involves both ripening and the response of the fruit to variations in the microclimate (10) and/or light exposure (12). If this is true, some form of pre- or postharvest treatment to stimulate anthocyanin biosynthesis might be used in conjunction with synchronous ripening, attained by breeding, to take full advantage of the capacity of cranberries to accumulate anthocyanin. The accumulation of anthocyanin in cranberries during postharvest storage, with or without pretreatment with ethylene-releasing compounds, is well documented (2, 3, 5). Our data suggest that the 3rd strategy, breeding for synchronous color development, is a realistic objective. With 'Crowley', which approaches this target, about 70% of the berries examined in our study were classified as dark, as compared to 14–45% for all cultivars compared (12).

Each of these strategies except the 2nd (breeding for small berries) would appear to be more effective with late-maturing cultivars than with early maturing cultivars. This expectation follows from the reduced anthocyanin contents found in the former, which undoubtedly would have been greater had the harvest been delayed. However, in many locations, delaying the harvest would increase the risk of frost damage and also increase the cost of frost protection (3, 5). Perhaps earliness in color development, rather than a higher capacity to accumulate anthocyanin, is the characteristic that would best accomplish the aims of the first strategy.

We suggest that a breeding study be designed to test the feasibility of improving cranberry color by enhancing the traits of earliness, synchronous color development, and the capacity to accumulate anthocyanin, singly and in combination. We also suggest that further efforts be made to establish the biological limit of anthocyanin accumulation in cranberry fruit under optimal production conditions, and to determine the genetic and environmental components of this characteristic.

Literature Cited

1. Bramlage, W.L., R.M. Devlin, and J.M. Smagula. 1972. Effects of preharvest application of ethephon on 'Early Black' cranberries. J. Amer. Soc. Hort. Sci. 97:625–628.
2. Chandler, F.B. and I. Demoranville. 1958. Cranberry varieties of North America. Mass. Agr. Expt. Sta. Bul. 513.
3. Craker, L.E. 1971. Postharvest color promotion in cranberry with ethylene. HortScience 6:137–139.
4. Demoranville, I.E. 1976. Cranberry varieties, p. 83–84. In: The modern art of cranberry cultivation. Publ. 112. Coop. Ext. Serv., Univ. of Massachusetts, Amherst.
5. Devlin, R.M. and I.E. Demoranville. 1978. Influence of two ethylene-releasing compounds on anthocyanin formation, size, and yield in 'Early Black' cranberries. Proc. 32nd Annu. Mtg. Northeast Weed Sci. Soc. 108–112.
6. Doughty, C.C. and R. Garren, Jr. 1970. 'Crowley', a new, early maturing cranberry variety for Washington and Oregon. Fruit Var. Hort. Dig. 24:88–89.
7. Eck, P. 1972. Cranberry yield and anthocyanin content as influenced by ethephon, SADH, and malathion. J. Amer. Soc. Hort. Sci. 97:213–214.
8. Francis, F.J. and G.J. Servadio. 1963. Relation between color of cranberries and color and stability of juice. Proc. Amer. Soc. Hort. Sci. 83:406–415.
9. Galletta, G.J. 1975. Blueberries and cranberries, p. 154–196. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

10. Hall, I.V. and R. Stark. 1972. Anthocyanin production in cranberry leaves and fruit, related to cool temperatures at a low light intensity. Hort. Res. 12:183–186.
11. McClure, J.W. 1975. Physiology and functions of flavonoids. p. 971–988. In: J.B. Harborne, T.J. Mabry, and H. Mabry (eds.), The flavonoids, Academic, New York.
12. Rigby, B., M.N. Dana, and L.K. Binning. 1972. Ethephon sprays and cranberry fruit color. HortScience 7:82–83.
13. Sapers, G.M., G.R. Graff, J.G. Phillips, and K.H. Deubert. 1986. Factors affecting the anthocyanin content of cranberry. J. Amer. Soc. Hort. Sci. 111:612–622.
14. Sapers, G.M., S.B. Jones, and G.T. Maher. 1983. Factors affecting the recovery of juice and anthocyanin from cranberries. J. Amer. Soc. Hort. Sci. 108:246–249.
15. Sapers, G.M., J.G. Phillips, H.M. Rudolf, and A.M. DiVito. 1983. Cranberry quality: selection procedures for breeding programs. J. Amer. Soc. Hort. Sci. 108:241–246.
16. Schmid, P. 1977. Long term investigation with regard to the constituents of various cranberry varieties (*Vaccinium macrocarpon* Ait.). Acta Hort. 61:241–254.
17. Servadio, G.J. and F.J. Francis. 1963. Relation between color of cranberries and color and stability of sauce. Food Tech. 17:124–128.
18. Swanson, B.G. and K.G. Weckel. 1975. Refrigerated storage of fresh cranberries. J. Food Sci. 40:259–261.
19. Weiss, J., K. Klein, H. Samann, and H. Gartner. 1977. Ergebnisse von untersuchungen uber die gehalte einiger inhaltsstoffe von cranberries (Vaccinium macrocarpon Ait.) sowie die verar beitung der beeren zu saft. Mitteilungen Klosterneuburg 27:83–86.
20. Zuckerman, B.M., I.E. Demoranville, F.J. Francis, K. Hayes, R.L. Norgren, S. Regling, C.W. Miller, and S.M. Paracer. 1966. Pigment and viscosity of juice and sauce of several cranberry varieties. Proc. Amer. Soc. Hort. Sci. 89:248–254.